From the INTERNATIONAL BUREAU

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 03 August 2000 (03.08.00)	
International application No. PCT/US99/28975	**Applicant's or agent's file reference** A-574
International filing date (day/month/year) 08 December 1999 (08.12.99)	**Priority date** (day/month/year) 09 December 1998 (09.12.98)
Applicant SIMONET, William, Scott et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

07 July 2000 (07.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Forax
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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
A-574		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/28975	08/12/1999	09/12/1998
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant AMGEN INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 25 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/07/2000	08.12.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bretherick, J Telephone No. +49 89 2399 8415

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/28975

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-3,7,8,11-13,15, 16,18-22,24-71,73, 76,78,81-84, 86-99	as originally filed			
4-6,9,10,14,17,23, 72,74,75,77,79,80, 85	as received on	12/07/2000	with letter of	06/07/2000

Claims, No.:

1-34	as received on	12/07/2000	with letter of	06/07/2000

Drawings, sheets:

1/24-24/24	as originally filed

Sequence listing part of the description, pages:

4,5,6,9,10,14,17,23,72,74,75,77,79,80,85,100-109, referring to Figures 1-8 and 18 of the application as originally filed,, filed with the letter of 06/07/2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-34
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-34
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-34
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

1. Regarding Part V, Art. 33 PCT:

a. Beloh et al. (1998) Neuron Vol. 21 pp. 1291-1302 disclose the amino acid sequences derived from murine and human genomic DNAs and cDNAs of artemin, a GDNF family member which supports peripheral and central neurons and binds to the GFRα3-RET receptor complex for its signalling function. Figure 3A and B show the amino acid sequence, the human of which is compared in Figure 3A to various members of the GDNF family.

b. The amino acid sequences have homologies falling within the definitions of sequences named in Figures 3, 7 and 18. Artemin has been synthesised, as evidenced by the neuronal growth studies involving a solution of recombinantly expressed artemin to test the pharmacological effects thereof (see also paragraph bridging pages 1299 and 1300 "production of recombinant Artemin protein" (production in E. coli).

It would appear that this document was not available to the public until after the valid priority date of the current application. The subject-matter of all claims is thus new under Art. 33(1)(2) PCT. Were the above document made available to the public before the current priority date, then the subject-matter of claims 1, 3-16, 19-25 and 33-34 would not be new.

c. Moreover, claim 2 for the glycosylated form of artemin, would be obvious in view of its eukaryotic origin. Claims 26-30, for antibodies binding to peptides comprising amino acid sequences of Figure 3, 7 or 18 and hybridomas related thereto would also be considered to lack an inventive step under Art. 33(1)(3) PCT.

d. WO97/33912 discloses GDNF and therapeutic use thereof. It also advocates the use of cells capable of secreting recombinant GDNF in semipermeable implantations (paragraph bridging pages 41 and 42). Since the above disclosure of artemin represents another variant of the GDNF family, the provision of cells according to claims 16-18, suitable for human implantation, as well as the devices of claims 31 and 32, for encapsulated cells in a semipermeable membrane would be obvious to the skilled person in the light of WO97/33912 and Beloh et al. supra.

An inventive step under Art. 33(1)(3) EPC would therefore not be accordable.

2. Regarding Part VI, R. 70.10 PCT, the following documents are cited:

a. WO00/01815 published on 13/01/2000, filing date 5/07/2000, with earliest priority date 6/07/98 and most recent 2/07/1999; of relevance are murine sequences SEQ IDs 15 and 16 and human sequences homologs SEQ IDs. 3 and 4.

b. WO00/04050 Published 27/01/2000, filing date 14/07/1999, priority (earliest 14/07/98, most recent 08/06/199; the long splice sequence variant of "enovin" being of relevance.

c. WO 0017360, Published 30/03/2000, filing date 22/09/1998, priority 22/09/1997. The sequence shown in Fig. 41 corresponds to amino acids 20-229 of GRNF4.

of neurodegenerative conditions such as the degeneration of the dopaminergic neurons of the substantia nigra in Parkinson's disease. The only current treatments for Parkinson's disease are palliative, aiming at increasing dopamine levels in the striatum. The expected impact of GDNF therapy is not simply to produce an increase in the dopaminergic neurotransmission at the dopaminergic nerve terminals in the striatum (which will result in a relief of the symptoms), but also to slow down, or even stop, the progression of the degenerative processes and to repair the damaged nigrostriatal pathway and restore its function. GDNF may also be used in treating other forms of damage to or improper function of dopaminergic nerve cells in human patients. Such damage or malfunction may occur in schizophrenia and other forms of psychosis. Current treatments for such conditions are symptomatic and require drugs which act upon dopamine receptors or dopamine uptake sites, consistent with the view that the improper functioning of the dopaminergic neurons which innervate these receptor-bearing neuronal populations may be involved in the disease process. In spite of the continued discovery of neurotrophic factors and the continuing research involving therapeutic compositions in this field, compounds for the treatment of nerve damage and/or the enhancement of proper nerve function are still needed.

SUMMARY OF THE INVENTION

The neurotrophic factor polypeptides of the present invention are designated herein as GDNF-related neurotrophic factor 4 (GRNF4) protein products, denoting the status as a fourth member of what has been referred to as the GDNF family of structurally related neurotrophic factors. The novel molecules are functionally characterized by the ability to bind GDNF family receptor-alpha-3 (GFRα-3) (*i.e.*, GRNF4 activity). The novel proteins and polypeptides also provide part of a molecular complex which mediates or induces phosphorylation of tyrosine residues of the Ret receptor protein tyrosine kinase. Exemplary GRNF4 protein products comprise an amino acid sequence selected from the group consisting of: an amino acid sequence of Figure 3 (SEQ ID NO. 7), an amino acid sequence of Figure 7 (SEQ ID NO. 11) and consensus sequences such as those depicted in

Figure 18 (SEQ ID NO. 12).

In one aspect, the present invention provides for the production of GRNF4 protein products by a means other than separation of the protein from a naturally occurring source. Such means include recombinant or genetic engineering techniques or chemical synthesis techniques. In alternative embodiments, the GRNF4 protein products are produced by a combination of genetic engineering and chemical techniques. It will be appreciated, however, that the mere separation of this previously unidentified molecule from its natural or native state to a purified and isolated state is also unique to the present invention.

"Naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

In another aspect of the present invention, the GRNF4 protein products may be made in glycosylated or non-glycosylated forms. Derivatives of GRNF4 proteins and polypeptides typically involve attaching a GRNF4 molecule to a water soluble polymer. For example, a GRNF4 protein or polypeptide may be conjugated to one or more polyethylene glycol molecules to decrease the precipitation of the GRNF4 protein product in an aqueous environment.

Yet another aspect of the present invention includes the various polynucleotides encoding GRNF4 protein products. The term "isolated polynucleotide(s)" or "isolated polynucleotide molecule(s)"as used herein refers to a polynucleotide which encodes a GRNF4 protein product but is in a form not found in nature, *e.g.*, a form suitable for use in genetically engineering a cell to express the protein product or a chemically synthesized polynucleotide encoding a GRNF4 protein product. These polynucleotides are used in the expression of GRNF4 in eukaryotic or prokaryotic host cells, wherein the expression product or a derivative thereof is characterized by the ability to bind GFRα-3, and to act as part of a molecular complex which mediates or induces phosphorylation of tyrosine residues of the Ret receptor protein tyrosine kinase. The isolated polynucleotides and/or the vectors or genetically engineered host cells containing these polynucleotides may be used for *in vitro* protein production as well as in cell therapy or gene therapy applications.

Suitable polynucleotides include those specifically depicted in the Figures as well as degenerate sequences, naturally occurring allelic variations and modified sequences based on the present invention. Exemplary polynucleotide molecules include: (a) sequences set forth in Figure 2 (SEQ ID NO. 6) or Figure 6 (SEQ ID NO. 10); (b) a molecule which (1) hybridizes to a complementary sequence of (a) and (2) encodes an amino acid sequence with GRNF4 activity; and (c) a molecule which but for the degeneracy of the genetic code would hybridize to a complementary sequence of (a) and (2) encodes an amino acid sequence with GRNF4 activity. Also disclosed herein are vectors containing such polynucleotides, wherein the sequences typically are operatively linked to one or more operational elements capable of effecting the amplification and/or expression of the sequence. Both prokaryotic and eukaryotic host cells containing such vectors are contemplated. Typically, the host cell is selected from mammalian cells and bacterial cells, such as a COS-7 cell or *E coli*, respectively. The present invention further includes the recombinant production of GRNF4 protein products wherein transformed or transfected host cells are grown in a suitable nutrient medium, and the GRNF4 protein product expressed by the cells is, optionally, isolated from the host cells and/or the nutrient medium. If bacterial expression is involved, the method may further include the step of refolding the neurotrophic factor. "Transformed or transfected" as used herein refers to cells that are no longer in their naturally occurring form, *i.e.*, the cells have been recombinantly or genetically engineered or modified to express the GRNF4 protein or polypeptide. Transformation of the cells may take place *in vivo* or *in vitro*, *e.g.*, the modification of isolated host cells. It will be appreciated by those skilled in the art that isolated host cells may be genetically engineered for use in both gene therapy and *in vitro* protein production.

The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the

BRIEF DESCRIPTION OF THE FIGURES

Figure 1a depicts a nucleotide (SEQ ID NO. 1) encoding an open reading frame of a clone (smcb2-00011-d2) which showed homology to the C-terminal active domain of GDNF.

Figure 1b depicts a comparison of the smcb2-00011-d2 open reading frame (SEQ ID NO. 2) to neurturin (SEQ ID NO. 3).

Figure 1c depicts the full sequence of murine GRNF4 (SEQ ID NOs. 4 and 5) which was obtained by further sequencing the smcb2-00011-d2 clone. The sequence includes GDNF-like homology and 3'-UTR.

Figure 2 depicts a polynucleotide molecule comprising a nucleotide sequence (SEQ ID NO. 6) encoding murine GRNF4. The amino acid sequence of a full length GRNF4 protein product is encoded by nucleotides 217 to 891.

Figure 3 depicts the 224 amino acid sequence (SEQ ID NO. 7) of the full length murine GRNF4 protein product.

Figure 4 depicts a comparison of murine GRNF4 (SEQ ID NO. 7)and neurturin (SEQ ID NO. 3) amino acid sequences. Murine GRNF4 is approximately 39% identical to neurturin

Figure 5 depicts a comparison of the murine GRNF4 amino acid sequence (SEQ ID NO. 7 to those of neurturin (SEQ ID NO. 3), persephin (SEQ ID NO. 9) and GDNF (SEQ ID NO. 8).

Figure 6 depicts the nucleotide sequence for human GRNF4 (SEQ ID NO. 10).

Figure 7 depicts the amino acid sequence for human GRNF4 (SEQ ID NO. 11).

Figure 8 depicts an amino acid sequence comparison between mouse (SEQ ID NO. 7) and human GRNF4 (SEQ ID NO. 11).

Figure 9 depicts a Northern blot analysis of human tissues for human GRNF4.

Figure 10 depicts a Northern blot analysis of mouse tissues for mouse GRNF4.

Figure 11 presents a radioautograph of (^{125}I)-labeled GRNF4 fractionated by a 16% SDS-PAGE under non-reducing (NR) and reducing conditions.

Figure 12 depicts the binding of (^{125}I)-labeled GRNF4 to the surface of NSR-5 cells (genetically engineered mouse neuroblastoma, Neuro-2a, cells that express GFRα-3).

Figure 13 depicts binding of GRNF4 to BiaCore surface coated by a soluble flag-tagged GFRα-3 protein.

Figure 14 depicts chemical cross-linking of (^{125}I)-labeled GRNF4 to the soluble GFRα-3-human Fc fusion protein.

Figure 15 depicts chemical cross-linking of (^{125}I)-labeled GRNF4 to GFRα-3 and Ret receptors expressed in NSR-5 cells.

Figure 16 depicts GRNF4 induced tyrosine phosphorylation of the Ret receptors expressed in NSR-5 cells.

Figure 17 depicts the dose-dependence of GRNF4 induced tyrosine phosphorylation of the Ret receptors expressed in NSR-5 cells (Figure 17, panel A) and the kinetics of GRNF4 induced tyrosine phosphorylation of the Ret receptors expressed in NSR-5 cells (Figure 17, panel B).

Figure 18 depicts a GRNF4 consensus sequence (SEQ ID NO. 12).

Figure 19 illustrates that the expression of the GRNF4 transgene resulted in marked dysplasia of the adrenal medulla and an adjacent paraganglion, both of which are

binding of GRNF4 proteins and polypeptides (and protein products) to GFRα-3. In addition, the binding of the novel GRNF4 proteins and polypeptides to GFRα-3 induces tyrosine autophosphorylation or activation of the Ret receptor protein tyrosine kinase. Using the present disclosure, it is well within the ability of those of ordinary skill in the art to determine whether a GRNF4 protein product has a biological activity equivalent to that of the mouse and human GRNF4 molecules set forth in the Figures.

As used herein, the term "GRNF4 nucleic acids" or "GRNF4 polynucleotide" when used to describe a polynucleotide molecule refers to a polynucleotide molecule or fragment thereof that:

a) comprises a nucleotide sequence as set forth in Figures 2 or 6 (SEQ ID NOS. 6 or 10);

b) has a nucleotide sequence encoding a protein product comprising an amino acid sequence that is at least 77 percent identical to the protein product encoded by a polynucleotide sequence of mouse or human GRNF4 as described herein, but may have a sequence anywhere from 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90,91, 92, 93, 94, 95, 96, 97 or 98 to 99 percent identical to the protein product encoded by a polynucleotide sequence of mouse or human GRNF4 as described herein;

c) is a naturally occurring allelic variant or alternate splice variant of (a) or (b);

d) is a nucleic acid variant of (a)-(c) produced as provided for herein;

e) has a sequence that is complementary to (a)-(d);

f) hybridizes to any of (a)-(e) under conditions of high stringency and/or

g) has a nucleotide sequence encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or 25 amino acid substitutions, additions and/or deletions of any mature human GRNF4 protein product (*i.e.*, an GRNF4 protein product with its endogenous signal peptide removed).

"Identity," as known to those skilled in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. As used herein, the terms "identical", "identity" or "percent identical" refer to a measure of the percent of identical matches between two or more sequences with gap alignments addressed by the particular algorithm. "Similarity" is a related concept, but in contrast to "identity", it measures both

choices to be made will depend on the specific comparison to be made, such as DNA to DNA, protein to protein, protein to DNA; and additionally, whether the comparison is between pairs of sequences (in which case GAP is generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

As demonstrated by a comparison of the mouse and human GRNF4 of the Figures, the sequences are 78.7% similar and 77.4% identical over the full length molecule. As demonstrated by the comparison of the amino acid sequences of mouse (SEQ ID NO. 7) and human (SEQ ID NO. 11) GRNF4 (Figure 8), the percent identity of mature forms of the molecule may be as high as 82 or 83 to 92% identical. Therefore, one skilled in the art will appreciate that a polynucleotide encoding a protein that has a 77%, or greater, identity as compared to human GRNF4 is recognized as a GRNF4 molecule. Protein products that are at least 82 percent identical (*e.g.*, using the GAP program) will typically have several amino acid substitutions, deletions, and/or insertions as compared with any of the wild type GRNF4. Usually, the substitutions of the native residue will be either alanine, or a conservative amino acid so as to have little or no effect on the overall net charge, polarity, or hydrophobicity of the protein. Possible substitutions are set forth in Table I.

Table I

Amino Acid Substitutions

Conservative:

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Uncharged Polar:	glutamine
	asparagine
	serine
	threonine
	tyrosine

amino acids 109-224	amino acids 89-228
amino acids 112-224	amino acids 113-228
amino acids 119-224	amino acids 116-228
amino acids 129-224	amino acids 133-224

Thus, it is contemplated that any or all of the residues from 1 through 80 to 1 through 132 may be removed from a GRNF4 without affecting binding to GFRα-3. Using known analysis techniques, it is further contemplated that C-terminal truncations may include the removal of one or more amino acid residues up to the last cysteine residue. Thus, GRNF4 protein products that are truncated forms of the molecule also may include the deletion of amino acid residues from either or both termini. Additional GRNF4 protein products are contemplated as involving a non-natural consensus sequence comprising as depicted in Figure 18 (SEQ ID NO. 12), *i.e.*, deleting, adding and/or substituting one or more amino acid residues to form consensus sequences, as based upon mouse and human GRNF4. Possible conservative, preferred and exemplary additions and substitutions are described above.

The present GRNF4 protein products and polynucleotides may be used for methods of treatment, or for methods of manufacturing medicaments for treatment. Such treatment includes the treatment of conditions responsive to the binding of GRNF4 to GFRα-3 and the activation of Ret receptor protein tyrosine kinase through GFRα-3.

Other aspects and advantages of the present invention will be apparent to those skilled in the art. For example, additional uses include new assay systems, transgenic animals and antibody production.

Study Models

The present invention provides for assay systems in which GRNF4 protein product activity may be detected by measuring an elicited physiological response in a cell or cell line which expresses GFRα-3 and Ret. A physiological response may comprise a biological effect similar to that of GDNF or neurturin, including but not limited to, enhanced dopamine uptake, extension of neurites, increased cell survival or growth, as well as the transcriptional activation of certain nucleic acid sequences (*e.g.*

ligated into pSport I (Product No. 18248-013, GIBCO BRL) cut with SalI- Not I (included in the kit). Ligated DNA was electroporated into Electromax DH10B cells (Product No.18290-015, GIBCO BRL). The average insert size was 2.3kb.

Example 2

Identification and Isolation of murine GRNF4

Following rearray of the OPG knockout crushed bone library and subtraction with common cDNA probes, several thousand clones from the library were sequenced from the 5' end. For subtraction, cDNA from some of the library's most abundant clones was used to make radioactive probes for hybridization to filters containing an array of 34.000 library clones. Select clones that did not hybridize to these probes were sequenced. One clone, smcb2-00011-d2 (Figure 1a, SEQ ID NO. 1), encoded an open reading frame (ORF) which showed homology to the C-terminal active domain of GDNF. All seven cysteine residues characteristic of TGF-β family members were present in the predicted open reading frame encoded by smcb2-00011-d2. The predicted ORF was 47% identical to neurturin, which is structurally related to GDNF (Figure 1b, SEQ ID NO. 2). An RXXR (Arg-Xaa-Xaa-Arg) cleavage site (commonly found in TGF-β family members) was also present, and thus, the expressed sequence tag (EST) was predicted to encode the sequence of the active portion of a novel GDNF family member. After confirmation of the double stranded sequence, the molecule was designated GRNF4 (for GDNF-related neurotrophic factor 4).

The 3' untranslated region of GRNF4 was obtained by further sequencing the smcb2-00011-d2 clone (Figure 1c, SEQ ID NO. 4). The 5' end of GRNF4 was cloned by 5' Rapid Amplification of cDNA Ends (*i.e.*, RACE, as described in Frohman, M.A. (1993) Methods of Enzymology 218:340-358) from the smcb2-00011-d2 clone. Four oligonucleotides were synthesized based on the cDNA sequence of the smcb2-00011-d2 clone. These oligonucleotides were used to screen potential sources for 5'RACE to obtain the full length coding region of GRNF4.

Oligonucleotide Probe	Sequence
2037-99	5-TCC GAC GAG CTG ATA CGT TTC C-3

The initial RACE product was nested with oligos 2038-01 + AP2 using the same reaction conditions described above (+ or -) 5µl 0.5M GC Melt (Clontech, Product No. 8419-1) with adjusted water volume. Following gel electrophoresis of the nested PCR products, bands at approximately 900bp and 1500bp appeared with both + or - GC melt. The bands were extracted, and the DNA was purified with the Qiaquick Gel Extraction Purification Kit (Qiagen, Product No. 28704).

Following verification of the fragments with internal check PCR, the fragments were subcloned into a PCR cloning plasmid (*i.e.*, pCR2.1 TOPO-TA Cloning Kit, Invitrogen, Product No. K4500-40) following manufacturer instructions. Following transformation of bacteria, clones were purified with the Spin Miniprep Kit (Qiagen, Product No. 27104) following manufacturer instructions. Clones were subjected to an internal check PCR using oligonucleotide pair (2037-99 + 2038-01) with PCR conditions noted above, and EcoRI Digest (0.5ug DNA, 2µl 10xBuffer H, 1µl EcoRI (10u/µl, Boehringer Mannheim, Indianapolis, IN. USA, Product No. 703 737), dH_2O to 20µl.) DNA was digested by incubating at 37°C for 30 minutes. Digested DNA was analyzed on 1%Agarose/1xTBE (GIBCO BRL, Product No. 15510-027).

Clones were submitted for sequencing. Several clones were obtained which extended the 5' end of the original EST. The sequence for the novel gene is depicted in Figure 2 (SEQ ID NO. 6). The starting Met (predicted ORF) starts at bp position 217. The predicted 224 amino acid ORF encoded by this full length murine GRNF4 gene contained a predicted signal peptide with an upstream stop. Within the GDNF family, full length GRNF4 was most highly related to neurturin, being approximately 38 % identical at the amino acid level. A comparison of murine GRNF4 (SEQ ID NO. 7) and neurturin (SEQ ID NO. 3) amino acid sequences is depicted in Figure 4. A comparison of murine GRNF4 amino acid sequence (SEQ ID NO. 7) to those of neurturin (SEQ ID NO. 3), persephin (SEQ ID NO. 9) and GDNF (SEQ ID NO. 8) is depicted in Figure 5. The relative sizes of the proteins are as follows:

GDNF murine:	241aa
Neurturin murine:	196aa
Persephin murine:	157aa
GRNF4 murine:	224aa

Example 3

Identification and Isolation of human GRNF4

The putative coding region of mouse clone, smcb2-00011-d2 (Example 2, Figure 1A, SEQ ID NO. 1), was used to search public databases for homologous sequences. A homologous sequence is defined as a sequence with sufficient identity (>20-25% at the amino acid level) to suggest that the sequences are related, (*i.e.* the genes encoding the sequences are derived from duplication of a common ancestor gene). Two human genomic clones (Accession Nos. AC005038 and AC005051) contained regions that are highly homologous to smcb2-00011-d2 (*i.e.*, 83% or greater identity (BLASTP) at amino acid level, representing the nucleic acid homology between human and mouse GRNF4 over the region spanning the original EST). This region included the active portion of mouse GRNF4 as well as a 3' translation stop codon. Furthermore, about 400 bp upstream of this region was a region homologous to the 5' end of the mouse cDNA, indicating the presence of an intron. The translation start codon was found in this upstream region. Thus, regions surrounding the translation start and stop codons were identified based on homology to mouse cDNA sequence.

Several oligonucleotide primers surrounding the translation start and stop codons were designed to clone the human cDNA coding region by PCR method. The probes are presented in Table 2.

Table 2

Oligonucleotide Primers for Amplification of Human GRNF4 Gene

Oligonucleotide Probe	Sequence
2058-59	5-GGT GGG GGA ACA GCT CAA CAA T-3
2058-60	5-CAA CAA TGG CTG ATG GGC G-3
2020-27	5-GTA AGG GTC CAG TCT GCA AAG-3
2035-28	5-TCA GCC CAG GCA GCC GCA G-3

The nucleotide sequence for human GRNF4 is depicted in Figure 6 (SEQ ID NO. 10). The amino acid sequence for human GRNF4 is depicted in Figure 7 (SEQ ID NO. 11).

Figure 8 depicts a protein sequence comparison between mouse (SEQ ID NO. 7) and human (SEQ ID NO. 11) GRNF4. The comparison demonstrates that the mouse and human sequences are 78.7% similar and 77.4% identical over the full length coding region.

Example 4

Production of murine GRNF4

The DNA fragment encoding the mature form of GRNF4 was amplified using PCR. The oligonucleotides used as primers for this reaction were designed such that XbaI and XhoI restriction sites were placed at the 5' and 3' ends of the gene respectively. The amplified PCR product was digested with the appropriate enzymes and cloned into a plasmid or expression vector as described above under recombinant expression of GRNF4.

The GRNF4 plasmid was then transformed into an *E coli* host cell for expression of the GRNF4 protein. Following induction, expression of the GRNF4 protein can be visualized by SDS PAGE.

GRNF4 was expressed in inclusion bodies in *E coli*. Inclusion bodies were solubilized in 6M guanidine HCl, 50 mM Tris, 8 mM DTT for one hour at room temperature. The solubilized inclusion bodies were diluted 25 fold into 2 M urea, 50 mM Tris, 160 mM arginine, 3 mM cysteine, pH 8.5 and stirred overnight in the cold (4°C). The mixture was clarified by centrifugation, concentrated about 10 fold, and diluted 3 fold with 1.5 M urea, 5 mM Tris, pH 9. The resulting mixture was clarified by centrifugation, pH adjusted to 6.8 (with phosphoric acid) and loaded onto an ion exchange column (SP-Sepharose column, Amersham Pharmacia Biotech) equilibrated in 10 mM Na phosphate, 0.2 M arginine, pH 6.8. After loading and washing the column with the same buffer, the GRNF4 was eluted off the column using a gradient from 0 to 1 M NaCl in the same buffer. Peak fractions were pooled and pH adjusted to 4.5. Ammonium sulfate was added to 0.8 M, and the mixture was loaded onto a hydrophobic interaction chromatography column (Butyl Toyopearl chromatography column, TosoHaas, Montgomeryville, PA, USA) equilibrated in 10 mM Na acetate, 0.8 M ammonium sulfate. After loading and washing

on ice for 5 to 10 minutes, washed once with ice-cold washing buffer (Dolbeco Modified Eagle's Medium (GIBCO/BRL) containing 25 mM HEPES, pH 7.4) and incubated with 0.2 ml of binding buffer (washing buffer supplied with 2mg/ml BSA) containing 0.1 nM of (^{125}I)GRNF4 in the presence or absence of 50 nM of unlabeled GRNF4 at 4°C for four hours. Cells were washed four times with 0.5 ml ice-cold washing buffer and lysed with 0.5 ml of 1 M NaOH. The lysates were counted in a 1470 Wizard Automatic Gamma Counter.

The results of this analysis are presented in Figure 12. The experiment demonstrated that recombinant GRNF4 specifically binds to cells expressing GFRα-3. In addition, cells expressing higher levels of GFRα-3 (*e.g.*, NSR-5) bind GRNF4 more efficiently.

Example 7

GRNF4 Binding to BiaCore Surface Coated by Soluble GFRα-3 Protein

GRNF4 was found to specifically bind a surface coated by a soluble flag-tagged GFRα-3 receptor (a surface plasmon resonance analysis, BIACORE® biosensor-based analytical instrument for studying interactions, BiaCore AB, Uppsala, Sweden). GRNF4 did not specifically bind GFRα-1 or GFRα-2 receptor proteins. The results of this analysis are presented in Figure 13.

Example 8

Tissue Distribution of GRNF4 mRNA

Tissue distributions of GRNF4 in both mouse and human were studied using Northern blot analysis. The human GRNF4 probe was prepared by a PCR reaction from human genomic DNA (Clontech). The probe region corresponds to nucleotides 484 to 672 in Figure 6 (SEQ ID NO. 10). This fragment was generated using appropriate primers, cloned into pCR2.1 (Clontech), and sequence verified. 20 ng of EcoRI fragment from this clone was labeled with ^{32}P-dCTP using Rediprime II kit (Amersham). Human Multiple Tissue Northern Blot (Figure 9A, Clontech) and Human Multiple Tissue Northern Blot II (Figure 9B, Clontech)

were hybridized in 10 ml of Expresshyb solution with $4X10^6$ cpm/ml of probe at 60°C for 14 hours. The blots were washed twice in 0.5%SDS, 2XSSC solution for 30 minutes at room temperature. They were further washed three times in 0.1%SDS, 0.1XSSC for 30 minutes at 55°C. These membranes then were exposed under an X-Omat AR film (Kodak) for three days at -80°C. The probe detected two strong bands at 4.3 and 1.7 kB especially in placenta, pancreas, and prostate (Figure 9). Somewhat weaker expression was also observed in testis, ovary, small intestine, colon (mucosal line), kidney, and heart. Interestingly, only the 1.7 kB band was observed in kidney. More tissues were surveyed with Human RNA master blot (Clontech, data not shown), and the results confirmed the Northern blot analysis. Furthermore, additional expression signals were observed in pituitary gland, fetal kidney, fetal lung, and adult trachea.

A portion of mouse cDNA (from nucleotide 649 to 954 in Figure 2, SEQ ID NO. 6) was labeled with ^{32}P-dCTP in a PCR labeling reaction. The same hybridization and wash protocol was used as in human Northern blot analysis, except $4X10^6$ cpm/ml of probe was used to hybridize Mouse multiple tissue northern blot (Clontech, Figure 10). 1.4 and 1.0 bands were detected in testis. As in human expression analysis, Mouse RNA master blot (Clontech) was used to survey more tissues. Testis and uterus showed strong GRNF4 expression, whereas thyroid, prostate, and epididymus showed moderate expression.

Example 9

Chemical Crosslinking of GRNF4 with GFRα-3

In order to study the binding properties and molecular characteristics of GRNF4, chemical crosslinking experiments were performed. The experiments involved (^{125}I)GRNF4 linked to a soluble GFRα-3/human Fc fusion protein or linked to GFRα-3 and Ret receptors expressed on the surface of NSR-5 cells (described in Example 6).

For crosslinking using the soluble GFRα-3 receptors, either (^{125}I)GDNF or (^{125}I)GRNF4 was added to 1 ml of 1x conditioned media of 293T cells which express the GFRα-1, GFRα-2, or GFRα-3/hFc fusion proteins, to a final concentration of 2 nM. The soluble GFRα-3/hFc protein was transiently expressed using 293T cells (293 cells, ATCC CRL-1573 expressing the SV40 large T antigen; modified by and obtained from

human and mouse neurturin; mouse, human, and rat persephin; and mouse human and rat glial-derived neurotrophic factor. These three molecules make up the previously known members of the GDNF family of neurotrophic factors. These results suggest that GRNF4 is a novel member of the GDNF family, representing the fourth member of this family.

As seen from this data, a "smallest sum probability" value for significant matches indicating a related family member are from 4.2e-12 to 1e-20. The smallest sum probability for a protein equivalent to mouse or human GRNF4 would have a score of 4.9e-21 to 1.2e-241 (the later value obtained for a match of a human molecule, *e.g.*, neurturin, to itself).

Example 14

GRNF4 Consensus Sequence

A GRNF4 consensus sequence is depicted in Figure 18 (SEQ ID NO. 12). This sequence is based upon the comparison of the amino acid sequences of mouse and human GRNF4, wherein "Xaa" represents a deletion, addition or substitution of an amino acid residue.

Example 15

Transgenic Overexpression of GRNF4 Driven by Ubiquitous ß-actin Promoter

The full coding region of murine GRNF4 was subcloned into an expression vector placing it under the control of the human ß-actin promoter and enhancer for ubiquitous expression in transgenic mice. (Klebig-ML. *et al*, *PNAS USA* 92:4728-4732, 1995; Ray-P. *et al*, *Genes and Development* 5:2265-2273, 1991) The murine GRNF4 cDNA was used as a template to PCR amplify a SalI/BamHI Fragment to clone into the ß-actin expression vector. The oligonucleotides were designed such that a SalI restriction site and Kozac consensus (GCCACC) sequence directly precedes the ATG (start codon) of the GRNF4 cDNA, and a BamHI restriction site follows the stop codon in the 3' UTR.

CLAIMS

What is claimed is:

1. An isolated and purified protein product comprising an amino acid sequence selected from the group consisting of:

a) Figure 3 (SEQ ID NO. 7),

b) amino acid residues 81 through 224 of Figure 3 (SEQ ID NO. 7),

c) amino acid residues 109 through 224 of Figure 3 (SEQ ID NO. 7),

d) amino acid residues 112 through 224 of Figure 3 (SEQ ID NO. 7),

e) amino acid residues 119 through 224 of Figure 3 (SEQ ID NO. 7),

f) amino acid residues 129 through 224 of Figure 3 (SEQ ID NO. 7),

g) Figure 7 (SEQ ID NO. 11),

h) amino acid residues 81 through 228 of Figure 7 (SEQ ID NO. 11),

i) amino acid residues 89 through 228 of Figure 7 (SEQ ID NO. 11),

j) amino acid residues 113 through 228 of Figure 7 (SEQ ID NO. 11),

k) amino acid residues 116 through 228 of Figure 7 (SEQ ID NO. 11),

l) amino acid residues 133 through 228 of Figure 7 (SEQ ID NO. 11),

m) Figure 18 (SEQ ID NO. 12),

n) amino acid residues PPP through CLG of Figure 18 (SEQ ID NO. 12),

o) amino acid residues AAR through CLG of Figure 18 (SEQ ID NO. 12),

p) amino acid residues AGXaa through CLG of Figure 18 (SEQ ID NO. 12), and

q) amino acid residues LRS through CLG of Figure 18 (SEQ ID NO. 12).

2. A protein product of Claim 1 which is glycosylated.

3. A protein product of Claim 1 which is non-glycosylated.

4. A pharmaceutical composition comprising a mixture of a protein product of claim 1, 2 or 3 and a pharmaceutically acceptable carrier.

5. An isolated polynucleotide molecule which encodes a protein product that is at

least 82% identical in amino acid sequence to a protein product of claim 1, wherein said protein product binds GDNF family receptor-alpha-3 (GFRα-3), and wherein said percent identity is determined by GAP, BLAST or FASTA using standard default parameters.

6. An isolated polynucleotide molecule which encodes a protein product that is at least 90% identical in amino acid sequence to a protein product of claim 1, wherein said protein product binds GDNF family receptor-alpha-3 (GFRα-3), and wherein said percent identity is determined by BLASTP using standard default parameters.

7. An isolated polynucleotide molecule selected from the group consisting of:

a) a molecule comprising the nucleotides of Figure 2 (SEQ ID NO. 6) or its complement,

b) a molecule encoding a polypeptide comprising amino acid residues 81 through 224 of Figure 3 (SEQ ID NO. 7),

c) a molecule encoding a polypeptide comprising amino acid residues 109 through 224 of Figure 3 (SEQ ID NO. 7),

d) a molecule encoding a polypeptide comprising amino acid residues 112 through 224 of Figure 3 (SEQ ID NO. 7),

e) a molecule encoding a polypeptide comprising amino acid residues 119 through 224 of Figure 3 (SEQ ID NO. 7),

f) a molecule encoding a polypeptide comprising amino acid residues 129 through 224 of Figure 3 (SEQ ID NO. 7),

g) a molecule comprising the nucleotides of Figure 6 (SEQ ID NO. 10) or its complement,

h) a molecule encoding a polypeptide comprising amino acid residues 81 through 228 of Figure 7 (SEQ ID NO. 11),

i) a molecule encoding a polypeptide comprising amino acid residues 89 through 228 of Figure 7 (SEQ ID NO. 11),

j) a molecule encoding a polypeptide comprising amino acid residues 113 through 228 of Figure 7 (SEQ ID NO. 11),

k) a molecule encoding a polypeptide comprising amino acid residues 116 through

228 of Figure 7 (SEQ ID NO. 11),

l) a molecule encoding a polypeptide comprising amino acid residues 133 through 228 of Figure 7 (SEQ ID NO. 11),

m) a molecule encoding a protein product comprising an amino acid sequence of Figure 3 (SEQ ID NO. 7),

n) a molecule encoding a protein product comprising an amino acid sequence of Figure 7 (SEQ ID NO. 11), and

o) a molecule encoding a protein product comprising an amino acid sequence of Figure 18 (SEQ ID NO. 12)

p) a molecule encoding a protein product comprising amino acid residues PPP through CLG of Figure 18 (SEQ ID NO. 12),

q) a molecule encoding a protein product comprising amino acid residues AAR through CLG of Figure 18 (SEQ ID NO. 12),

r) a molecule encoding a protein product comprising amino acid residues AGXaa through CLG of Figure 18 (SEQ ID NO. 12), and

s) a molecule encoding a protein product comprising amino acid residues LRS through CLG of Figure 18 (SEQ ID NO. 12).

8. An isolated polynucleotide molecule selected from the group consisting of:

a) a molecule which hybridizes under stringent conditions to a complementary sequence of a polynucleotide molecule of Claim 5; and

b) a molecule which but for the degeneracy of the genetic code would hybridize under stringent conditions to a complementary sequence of a polynucleotide molecule of Claim 5,

and wherein said isolated polynucleotide molecule encodes a protein product that binds GDNF family receptor-alpha-3 (GFRα-3).

9. A vector comprising a polynucleotide molecule according to claim 5, 6, 7 or 8.

10. A vector according to claim 9, further comprising one or more operational elements capable of effecting the amplification or expression of said polynucleotide molecule.

11. A vector comprising polynucleotide molecule selected from the group consisting of:

a) a molecule encoding a polypeptide comprising amino acid residues 81 through 224 of Figure 3 (SEQ ID NO. 7),

b) a molecule encoding a polypeptide comprising amino acid residues 109 through 224 of Figure 3 (SEQ ID NO. 7),

c) a molecule encoding a polypeptide comprising amino acid residues 112 through 224 of Figure 3 (SEQ ID NO. 7),

d) a molecule encoding a polypeptide comprising amino acid residues 119 through 224 of Figure 3 (SEQ ID NO. 7),

e) a molecule encoding a polypeptide comprising amino acid residues 129 through 224 of Figure 3 (SEQ ID NO. 7),

f) a molecule encoding a polypeptide comprising amino acid residues 81 through 228 of Figure 7 (SEQ ID NO. 11),

g) a molecule encoding a polypeptide comprising amino acid residues 89 through 228 of Figure 7 (SEQ ID NO. 11),

h) a molecule encoding a polypeptide comprising amino acid residues 113 through 228 of Figure 7 (SEQ ID NO. 11),

i) a molecule encoding a polypeptide comprising amino acid residues 116 through 228 of Figure 7 (SEQ ID NO. 11),

j) a molecule encoding a polypeptide comprising amino acid residues 133 through 228 of Figure 7 (SEQ ID NO. 11),

k) a molecule encoding a protein product comprising amino acid residues PPP through CLG of Figure 18 (SEQ ID NO. 12),

l) a molecule encoding a protein product comprising amino acid residues AAR through CLG of Figure 18 (SEQ ID NO. 12),

m) a molecule encoding a protein product comprising amino acid residues AGXaa through CLG of Figure 18 (SEQ ID NO. 12), and

n) a molecule encoding a protein product comprising amino acid residues LRS through CLG of Figure 18 (SEQ ID NO. 12).

12. A genetically engineered host cell comprising a polynucleotide molecule according

to claim 5, 6, 7 or 8.

13. An isolated host cell comprising a polynucleotide molecule according to claim 5, 6, 7 or 8.

14. A genetically engineered host cell which expresses a protein product comprising an amino acid sequence selected from the group consisting of:

a) an amino acid sequence of Figure 3 (SEQ ID NO. 7),
b) amino acid residues 81 through 224 of Figure 3 (SEQ ID NO. 7),
c) amino acid residues 109 through 224 of Figure 3 (SEQ ID NO. 7),
d) amino acid residues 112 through 224 of Figure 3 (SEQ ID NO. 7),
e) amino acid residues 119 through 224 of Figure 3 (SEQ ID NO. 7),
f) amino acid residues 129 through 224 of Figure 3 (SEQ ID NO. 7),
g) an amino acid sequence of Figure 7 (SEQ ID NO. 11),
h) amino acid residues 81 through 228 of Figure 7 (SEQ ID NO. 11),
i) amino acid residues 89 through 228 of Figure 7 (SEQ ID NO. 11),
j) amino acid residues 113 through 228 of Figure 7 (SEQ ID NO. 11),
k) amino acid residues 116 through 228 of Figure 7 (SEQ ID NO. 11),
l) amino acid residues 133 through 228 of Figure 7 (SEQ ID NO. 11),
m) an amino acid sequence of Figure 18 (SEQ ID NO. 12),
n) amino acid residues PPP through CLG of Figure 18 (SEQ ID NO. 12),
o) amino acid residues AAR through CLG of Figure 18 (SEQ ID NO. 12),
p) amino acid residues AGXaa through CLG of Figure 18 (SEQ ID NO. 12), and
q) amino acid residues LRS through CLG of Figure 18 (SEQ ID NO. 12),

and wherein said protein product is capable of binding GDNF family receptor-alpha-3 (GFRα-3).

15. A genetically engineered host cell comprising a vector of claim 10.

16. A genetically engineered host cell of Claim 12 wherein said cell is suitable for human implantation and wherein said cell expresses and secretes said polynucleotide molecule.

17. An isolated host cell of Claim 13 wherein said cell is suitable for human implantation.

18. An isolated host cell of Claim 16 or 17 wherein said cell is enclosed in a semipermeable membrane suitable for human implantation.

19. A method for the production of a protein product which is capable of binding to GDNF family receptor-alpha-3 (GFRα-3), said method comprising the steps of:

(a) culturing a host cell comprising an isolated polynucleotide molecule according to claim 5. 6. 7 or 8, under conditions suitable for the expression of said protein product by said host cell; and

(b) optionally, isolating said protein product expressed by said host cell.

20. A method for the production of a protein product which is capable of binding to GDNF family receptor-alpha-3 (GFRα-3), said method comprising the steps of:

(a) culturing an isolated host cell comprising a polynucleotide molecule encoding a protein product according to claim 1, under conditions suitable for the expression of said protein product by said host cell; and

(b) optionally, isolating said protein product expressed by said host cell.

21. A method of claim 19, wherein said polynucleotide molecule encodes a protein product comprising:

a) amino acid residues 81 through 224 of Figure 3 (SEQ ID NO. 7),

b) amino acid residues 109 through 224 of Figure 3 (SEQ ID NO. 7),

c) amino acid residues 112 through 224 of Figure 3 (SEQ ID NO. 7),

d) amino acid residues 119 through 224 of Figure 3 (SEQ ID NO. 7), or

e) amino acid residues 129 through 224 of Figure 3 (SEQ ID NO. 7).

22. A method of claim 19, wherein said polynucleotide molecule encodes a protein product comprising:

a) amino acid residues 81 through 228 of Figure 7 (SEQ ID NO. 11),
b) amino acid residues 89 through 228 of Figure 7 (SEQ ID NO. 11),
c) amino acid residues 113 through 228 of Figure 7 (SEQ ID NO. 11),
d) amino acid residues 116 through 228 of Figure 7 (SEQ ID NO. 11),
e) amino acid residues 133 through 228 of Figure 7 (SEQ ID NO. 11),
f) amino acid residues PPP through CLG of Figure 18 (SEQ ID NO. 12),
g) amino acid residues AAR through CLG of Figure 18 (SEQ ID NO. 12),
h) amino acid residues AGXaa through CLG of Figure 18 (SEQ ID NO. 12), or
i) amino acid residues LRS through CLG of Figure 18 (SEQ ID NO. 12).

23. An isolated and purified protein product prepared according to the method of claim 19.

24. An isolated and purified protein product prepared according to the method of claim 20.

25. A protein product which is capable of binding to GDNF family receptor-alpha-3 (GFRα-3), prepared by a method comprising the steps of:

(a) culturing a host cell containing a polynucleotide molecule selected from the group consisting of:

i) a molecule comprising the nucleotides of Figure 2 (SEQ ID NO. 6) or its complement,
ii) a molecule encoding a polypeptide comprising amino acid residues 81 through 224 of Figure 3 (SEQ ID NO. 7),
iii) a molecule encoding a polypeptide comprising amino acid residues 109 through 224 of Figure 3 (SEQ ID NO. 7),
iv) a molecule encoding a polypeptide comprising amino acid residues 112 through 224 of Figure 3 (SEQ ID NO. 7),
v) a molecule encoding a polypeptide comprising amino acid residues 119 through 224 of Figure 3 (SEQ ID NO. 7),
vi) a molecule encoding a polypeptide comprising amino acid residues 129 through 224 of Figure 3 (SEQ ID NO. 7),

vii) a molecule comprising the nucleotides of Figure 6 (SEQ ID NO. 10) or its complement,

viii) a molecule encoding a polypeptide comprising amino acid residues 81 through 228 of Figure 7 (SEQ ID NO. 11),

ix) a molecule encoding a polypeptide comprising amino acid residues 89 through 228 of Figure 7 (SEQ ID NO. 11),

x) a molecule encoding a polypeptide comprising amino acid residues 113 through 228 of Figure 7 (SEQ ID NO. 11),

xi) a molecule encoding a polypeptide comprising amino acid residues 116 through 228 of Figure 7 (SEQ ID NO. 11),

xii) a molecule encoding a polypeptide comprising amino acid residues 133 through 228 of Figure 7 (SEQ ID NO. 11),

xiii) a molecule encoding a protein product comprising an amino acid sequence of Figure 3 (SEQ ID NO. 7),

xiv) a molecule encoding a protein product comprising an amino acid sequence of Figure 7 (SEQ ID NO. 11), and

xv) a molecule encoding a protein product comprising an amino acid sequence of Figure 18 (SEQ ID NO. 12)

xvi) a molecule encoding a protein product comprising an amino acid residues PPP through CLG of Figure 18 (SEQ ID NO. 12),

xvii) a molecule encoding a protein product comprising an amino acid residues AAR through CLG of Figure 18 (SEQ ID NO. 12),

xviii) a molecule encoding a protein product comprising an amino acid residues AGXaa through CLG of Figure 18 (SEQ ID NO. 12), and

xix) a molecule encoding a protein product comprising an amino acid residues LRS through CLG of Figure 18 (SEQ ID NO. 12)

under conditions suitable for the expression of said protein product by said host cell; and

(b) optionally, isolating said protein product expressed by said host cell.

26. An antibody that binds to a peptide comprising an amino acid sequence of Figure 3 (SEQ ID NO. 7), Figure 7 (SEQ ID NO. 11) or Figure 18 (SEQ ID NO. 12).

27. The antibody of claim 26 wherein said antibody is a monoclonal antibody.

28. The antibody of claim 26 wherein said antibody is a polyclonal antibody.

29. An antibody produced by immunizing an animal with a peptide comprising an amino acid sequence of Figure 3 (SEQ ID NO. 7), Figure 7 (SEQ ID NO. 11) or Figure 18 (SEQ ID NO. 12).

30. A hybridoma that produces a monoclonal antibody that binds to a peptide comprising an amino acid sequence of Figure 3 (SEQ ID NO. 7), Figure 7 (SEQ ID NO. 11) or Figure 18 (SEQ ID NO .12).

31. A device, comprising:

(a) a membrane suitable for implantation; and

(b) cells encapsulated within said membrane, wherein said cells secrete a protein product of claim 1;

said membrane being permeable to said protein product and impermeable to materials detrimental to said cells.

32. A device, comprising:

(a) a membrane suitable for implantation; and

(b) cells encapsulated within said membrane, wherein said cells contain a polynucleotide molecule selected from the group consisting of:

i) a molecule comprising the nucleotides of Figure 2 (SEQ ID NO. 6),

ii) a molecule comprising the nucleotides of Figure 6 (SEQ ID NO. 10),

iii) a molecule encoding a protein product comprising amino acid residues 81 through 228 of Figure 7 (SEQ ID NO. 11),

iv) a molecule encoding a protein product comprising amino acid residues 89 through 228 of Figure 7 (SEQ ID NO. 11),

v) a molecule encoding a protein product comprising amino acid residues 113 through 228 of Figure 7 (SEQ ID NO. 11),

vi) a molecule encoding a protein product comprising amino acid residues 116

through 228 of Figure 7 (SEQ ID NO. 11),

vii) a molecule encoding a protein product comprising amino acid residues 133 through 228 of Figure 7 (SEQ ID NO. 11),

viii) a molecule encoding a protein product comprising amino acid residues PPP through CLG of Figure 18 (SEQ ID NO. 12),

ix) a molecule encoding a protein product comprising amino acid residues AAR through CLG of Figure 18 (SEQ ID NO. 12),

x) a molecule encoding a protein product comprising amino acid residues AGXaa through CLG of Figure 18 (SEQ ID NO. 12), and

xi) a molecule encoding a protein product comprising amino acid residues LRS through CLG of Figure 18 (SEQ ID NO. 12),

wherein said cells express and secrete said protein product,

and wherein said membrane is permeable to said protein product and impermeable to materials detrimental to said cells.

33. The use of the isolated and purified protein product of claim 1 for the manufacture of a pharmaceutical composition.

34. A pharmaceutical composition comprising a protein product of claim 1 in combination with a pharmaceutically acceptable carrier.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
A-574		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 28975	08/12/1999	09/12/1998
Applicant		
AMGEN INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   GRNF4, A GDNF-RELATED NEUROTROPHIC FACTOR

5. With regard to the **abstract**,

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

On line 1 : delete "novel" before "molecules"

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/US 99/28975

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/475 C07K16/22 C12N5/10 C12N5/12
A61K38/18 A61K35/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BALOH R. H. ET AL.: "ARTEMIN, A NOVEL MEMBER OF THE GDNF LIGAND FAMILY, SUPPORTS PERIPHERAL AND CENTRAL NEURONS AND SIGNALS THROUGH THE GFRALPHA3- RET RECEPTOR COMPLEX" NEURON, vol. 21, no. 6, December 1998 (1998-12), pages 1291-1302, XP000857438	1-25,33, 34
Y	the whole document	26-32
Y	WO 97 33912 A (GENENTECH INC ;KLEIN ROBERT D (US); MOORE MARK W (US); ROSENTHAL A) 18 September 1997 (1997-09-18) page 15, line 20 - line 25	26-32

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2000	25/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mandl, B

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	WO 00 01815 A (BLOM NIKOLAJ ;HANSEN CLAUS (DK); JOHANSEN TEIT E (DK); NEUROSEARCH) 13 January 2000 (2000-01-13) the whole document, in particular SEQ.IDs.3 and 4 and SEQ.IDs. 15 and 16 ---	1-30,33, 34
E	WO 00 04050 A (GEERTS HUGO ALFONSO ;MASURE STEFAN LEO JOZEF (BE); CIK MIROSLAV (B) 27 January 2000 (2000-01-27) figures 21-23 ---	1-30,33, 34
E	WO 00 17360 A (SZKUDLINSKI MARIUSZ W ;WEINTRAUB BRUCE D (US); UNIV MARYLAND (US)) 30 March 2000 (2000-03-30) page 185 -page 188; figure 41 ---	1,3,4, 23-25, 33,34
P,X	MASURE S. ET AL.: "ENOVIN, A MEMBER OF THE GLIAL CELL-LINE-DERIVED NEUROTROPHIC FACTOR (GDNF) FAMILIY WITH GROWTH PROMOTING ACTIVITY ON NEURONAL CELLS" EUROPEAN JOURNAL OF BIOCHEMISTRY,DE,BERLIN, vol. 266, no. 3, 1999, pages 892-902, XP000882986 ISSN: 0014-2956 the whole document -----	1-30,33, 34

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
US 99/28975

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9733912 A	18-09-1997	AU 719482 B	11-05-2000
		AU 2217297 A	01-10-1997
		CA 2246768 A	18-09-1997
		EP 0888385 A	07-01-1999
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(57) Abstract

The present invention relates to molecules referred to as GDNF-related neurotrophic factor 4 (GRNF4). The present invention involves the cloning, expression, characterization and use of GRNF4. Polynucleotide molecules and amino acid sequences are described for GRNF4. GRNF4 is 35 % identical to glial cell line-derived neurotrophic factor and 46 % identical to neurturin. GRNF4 binds to GFRα-3 which is a receptor of the GFRα family exclusively expressed in the peripheral sensory and sympathetic nervous systems.

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A. CLASSIFICATION OF SUBJECT MATTER
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A61K38/18 A61K35/12

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Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
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19 July 2000	25/07/2000

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European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mandl, B

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